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Isolation and Characterization of a New Cyanobacterial Strain with a Unique Fatty Acid Composition

Fariza K. Sarsekeyeva[1,2], Aizhan A. Usserbaeva[2], Bolatkhan K. Zayadan[2], Kirill S. Mironov[1], Roman A. Sidorov[1], Anna Yu. Kozlova[1], Elena V. Kupriyanova[1], Maria A. Sinetova[1], Dmitry A. Los[1*]

[1]Institute of Plant Physiology, Russian Academy of Sciences, Moscow, Russia
[2]Department of Biotechnology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan
Email: *losda@ippras.ru

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Abstract

A new cyanobacterial strain was isolated and purified from salt Lake Balkhash, Kazakhstan. According to its morphological and ultrastructural characteristics, 16S rRNA sequence and the fatty acid profile, the strain has been classified as *Cyanobacterium* spp. and assigned as *Cyanobacterium* sp. IPPAS B-1200. The strain is characterized by a non-temperature inducible Δ9-desaturation system, and by high relative amounts of myristic (14:0—30%) and myristoleic (14:1Δ9—10%) acids. The total amount of C_{14} fatty acids reaches 40%, which is unusually high for cyanobacteria, and it has never been reported before. The remaining fatty acids are represented mainly by palmitic (16:0) and palmitoleic (16:1Δ9) acids (the sum reaches nearly 60%). Such a fatty acid composition, together with a relatively high speed of growth, makes this newly isolated strain a prospective candidate for biodiesel production.

Keywords

Cyanobacterium, Fatty Acids, Lake Balkhash, Myristic Acid, Myristoleic Acid, 16S rRNA

1. Introduction

Cyanobacteria (formerly blue-green algae) are considered as one of the most ancient group of living organisms

*Corresponding author.

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on Earth [1]. Different species of modern cyanobacteria inhabit almost all environments—from soil to fresh and sea waters, as well as such extreme habitats as hot springs, soda and salt lakes, etc. Cyanobacteria, which are characterized by rapid photoautotrophic growth and high speed of biomass accumulation, are now considered as important renewable energy alternatives for petroleum-based fuels, *i.e.* biofuels—biogas [2], bioethanol [3] [4] butanol [5], or biodiesel [6].

Biodiesel is produced by trans-esterification of plant oils, yielding fatty acid methyl or ethyl esters. Such production depends on the availability of vegetable oil feedstock. CO_2-fixing and oil-producing eukaryotic algae might be another source to produce biodiesel. However, there are still technological barriers and economic issues that hamper the industrial development of high-yield oil-producing algal strains [7]. Cyanobacteria have several advantages in comparison to higher plants and algae: 1) they grow much faster than competitors; 2) they have high photosynthetic efficiency; 3) they are transformable by double homologous recombination. The latter allows targeted mutagenesis and genetic transformation that imply full-scale metabolic design and engineering of organisms that efficiently convert solar energy into high-value products [8].

Cyanobacteria usually do not store fatty acids (FAs) in the form of triacylglycerides. The majority of their FAs are a part of diacylglycerides of the membrane lipids. According to fatty acid composition, all cyanobacteria have been divided into four groups [9]. Organisms in Group 1 (mainly representatives of mesophilic and thermophilic *Synechococcus*) introduce only one double bond at the Δ9 position of fatty acids (usually C_{16} or C_{18} FAs) at the *sn-l* position. In cyanobacteria of Group 2 (*Anabaena*, *Nostoc*, *Gloeobacter violaceus*, etc.), the C_{18} acid is desaturated at the Δ9, Δ12, and Δ15 positions and the C_{16} acid is desaturated at the Δ9 and Δ12 positions. In Group 3 (*Spirulina* or *Arthrospira* strains), the C_{18} acid is desaturated at the Δ6, Δ9, and Δ12 positions. Finally, in Group 4 (model strain *Synechocystis* sp. PCC 6803), the C_{18} stearic acid is desaturated at the Δ6, Δ9, Δ12, and Δ15 positions. The C_{16} palmitic acid is not desaturated in Groups 3 and 4 [9].

Resent advances in sequencing techniques allow determination of the whole genomes of various cyanobacterial strains. Genes for specific fatty acid desaturases have been identified, and they raise the need of an update to the previously postulated FA-based classification of cyanobacteria. Now, at least, one additional group of cyanobacteria should be allocated, in which C_{18} and C_{16} fatty acids are desaturated at positions Δ9 and Δ12. The organisms in this group are represented by marine species of *Prochlorococcus* and *Synechococcus* [10], and they can produce dienoic fatty acids.

Control of FAs carbon chain length provides a mechanism to generate biofuels with specified properties, since $C_{10\text{-}14}$ FAs may be used as components of jet biofuels, whereas $C_{16\text{-}18}$ FAs may be employed for biodiesel production [11]. So far, $C_{10\text{-}12}$ FAs of cyanobacteria have been found in significant amounts (at 50% of the total FAs of *Trichodesmium erythraeum* grown in laboratory conditions) in an early report [12]. However, their abundant presence has not been confirmed in the same strain grown in natural environment [13]. C_{14} FAs have been reported in significant amounts in several strains: 14:0 in *Trichodesmium erythraeum* [11] [12] or *Phormidium* J-1 [14]; 14:0 and 14:1 in *Prochlorothrix hollandica* (up to 30%) [15]. The latter strain, however, is rather capricious and problematic for mass cultivation.

Here we report the isolation and partial characterization of a new strain, which belongs to cyanobacteria of Group 1 [9], and produces 40% of medium-chain C_{14} FAs together with nearly 60% of C_{16} FAs. This strain can efficiently grow in a wide range of temperatures. These unique characteristics put this cyanobacterium into a line of the potential producers of biofuels.

2. Materials and Methods

2.1. Collection, Purification, and Growth of Cyanobacterial Strain

Samples of cyanobacteria were from the Bay Kurta, Lake Balkhash, Republic of Kazakhstan (46˚32'27" North latitude, 74˚52'44" East longitude). Samples were collected in October 2013, when water temperature on the surface was 15˚C - 17˚C, and salinity of water was 6 $g \cdot l^{-1}$. The area of Lake Balkhash is 18,200 km^2; it is situated 342 m above sea level. For isolation and cultivation of the strains, standard methods were used. Algologically pure and axenic cultures were obtained by dilutions and plating on Zarrouk medium [16] [17], which was prepared as follows ($g \cdot l^{-1}$): $NaHCO_3$—16.8; $K_2HPO_4 \times 3H_2O$—1; $NaNO_3$—2.5; K_2SO_4—1; NaC1—1.0; $MgSO_4 \times 7H_2O$—0.2; $CaCl_2 \times 2H_2O$—0.027. Microelements №1 at 1 $ml \cdot l^{-1}$ ($g \cdot l^{-1}$): H_3BO_3—2.86; $MnCl_2 \times 4H_2O$—1.81; $ZnSO_4 \times 7H_2O$—0.22; $CuSO_4 \times 5H_2O$—0.08; MoO_3—0.015. Microelements №2 at 1 $ml \cdot l^{-1}$ ($g \cdot l^{-1}$): NH_4VO_3—0.023; $K_2Cr_2(SO_4)_4 \times 24H_2O$—0.096; $NiSO_4 \times 7H_2O$—0.048; $Na_2WO_4 \times 2H_2O$—0.018; $Ti_2(SO_4)_3$—0.040;

$Co(NO_3)_2 \times 6H_2O$ —0.044. Fe + EDTA solution—1 $ml \cdot l^{-1}$ (30.2 g EDTA-Na_2 was dissolved in 134 ml of 1M KOH. This solution was diluted with 500 ml of distilled H_2O, and 24.9 g of $FeSO_4 \times 7H_2O$ was added, and the volume was adjusted to 1:l with distilled H_2O (the latter solution requires overnight bubbling with air through NaOH solution to avoid reaction with CO_2). Solid Zarrouk medium contained 1% agar. The culture was grown under continuous illumination (20 - 50 μmoles photon $m^{-2} \cdot s^{-1}$) at 25˚C - 30˚C. Bacterial contamination was eliminated by the addition of antibiotics, ampicillin (25 - 50 $\mu g \cdot ml^{-1}$) or trimethoprim (5 - 30 $\mu g \cdot ml^{-1}$). Single colonies that appeared within one to two weeks were plated on fresh medium. Bacterial contamination was tested by growing single clones on a standard LB medium [18] at 30˚C - 37˚C for 12 - 72 h. No fungal or algal contamination was observed.

2.2. Light and Electron Microscopy

Morphology of strains was accessed by light microscopy (Axio Imager A1; Carl Zeiss, Germany). Morphological characterization and assignment was done as described previously [19]. Ultrastructure of cyanobacterial cells was analyzed by transmission electron microscopy. Cells from 25 ml of culture were collected by centrifugation at room temperature (4500 *g*, 5 min) and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 4˚C. Samples were rinsed three times in the same buffer and post-fixed in 1% OsO_4 for 1 h. After dehydration in ethanol and acetone, samples were embedded in Epon resin (Sigma-Aldrich, St. Louis, MO, USA). Cell sections were contrasted with uranyl acetate and then with lead citrate according to Reynolds [20] and analyzed with a Libra-120 transmission electron microscope.

2.3. Experimental Conditions of Cultivation

Intensive cultivation was carried out in a custom made installation (apparatus), developed in the laboratory, in glass vessels with 250 ml of Zarrouk medium. Cells were grown under continuous illumination with white luminescent lamps at 110 μmoles of photons $m^{-2} \cdot s^{-1}$, and aerated with sterile air-gas mixture that contained 1.5% - 2% of CO_2. To determine the optimal temperature for growth, cultures were incubated at four different temperatures: 24˚C ± 1˚C, 27˚C ± 1˚C, 32˚C ± 1˚C, and 39˚C ± 1˚C. All growth experiments were repeated, at least, 3 times.

2.4. DNA Isolation and Cloning of the 16S rDNA Fragment

Total genomic DNA was isolated from cyanobacterial cells by hot phenol [21]. Polymerase chain reaction (PCR) analysis was carried out with GenAmp 2720 thermocycler (Life Technologies, Grand Island, NY, USA). The DNA fragment that corresponded to the 16S rRNA was amplified from the genomic DNA with standard synthetic oligonucleotides used as forward and reverse primers for PCR:
5'-AGAGTTTGATCCTGGCTCAG-3'and5'-AAGGAGGTGATCCAGCC-3' [22]. Another pair of primers (5'-CGGACGGGTGAGTAACGCGTGA and 5-GACTACWGGGGTATCTAATCCCWTT) was used to confirm the presence of the amplified DNA fragments in the recombinant plasmids [23]. Fragments of DNA after purification were cloned in T-vector pTZ57R/T (Fermentas, Vilnius, Lithuania). Competent cells of *E. coli* strain XL1-Blue (Stratagene, La Jolla, CA, USA) were used for transformation together with the Transform Aid Bacterial Transformation Kit (Fermentas). Isolation of plasmid DNA from cells *E. coli* was carried out with Gene JET Plasmid Miniprep Kit (Fermentas). Nucleotide sequences of cloned DNA fragments were determined with automated capillary genetic analyzer ABI Prizm 310 (Life Technologies). Alignments of nucleotide sequences [24] were performed with BLAST (http://blast.ncbi.nlm.nih.gov). The determined nucleotide sequence was deposited in GenBank under Accession number KM502966.

2.5. Lipid Content and Fatty Acid Analysis

For determination of lipid content and fatty acid composition, samples were withdrawn at the stationary phase of culture that had been grown at 32˚C and 24˚C. Lipid content and fatty acid composition of the fixed samples of 15 - 50 ml (depending on the strain and the culturing conditions) were determined as follows. Cells were pelleted by centrifugation at room temperature for 3 min at 3600 *g*. The supernatant was removed, and pelleted cells were resuspended in 10 ml of distilled water, quantitatively transferred to 15 ml centrifuge tubes and pelleted again by centrifugation. The supernatant was removed again, and the precipitate was immediately resus-

pended in 7.5 ml of hot (60˚C) isopropyl alcohol that contained ionol at 0.02%. The tubes were incubated for 10 min in a water bath heated to 65˚C, then capped and stored until analysis at −20˚C. Fatty acid methyl esters (FAMEs) were prepared by transesterification of the stored materials in a mixture of methanol and acetyl chloride (9:1) for 60 min at 70˚C. Analysis of the resulting mixture of FAMEs and quantitative content of total lipids in terms of esterified fatty acids was performed with GC-MS Agilent 7890A gas-liquid chromatography system with the mass spectrometric detector Agilent 5975S (Agilent Technology Systems, Santa Clara, CA, USA). The 60-m capillary column DB-23 (∅ 0.25 mm; Fischer Scientific, Loughborough, UK) was filled with 50% cyanopropyl methylpolysiloxane. Other separation conditions for FAMEs were as follows: helium pressure in the injector was 245 kPa; flow rate of 1 ml·min^{-1}; volume of the sample solution was 1 µl; flow divider 1:5; and the evaporation temperature of 260˚C. Program of column gradient temperature: jump from 130˚C to 170˚C at 6.5˚C per min; jump from 170˚C to 215˚C at 2.75˚C per min; holding at 215˚C for 25 min, jump from 215˚C to 240˚C at 40˚C per min; and holding at 240˚C for 50 min. Operating temperature of MS detector was 240˚C, at the ionization energy of 70 eV. Heptadecanoic acid (17:0) was used as the internal standard.

3. Results

3.1. Isolation and Purification of the Cyanobacterial Strain

Water samples from Lake Balkhash were plated on BG11 [25] or Zarrouk [17] media. Single colonies were picked up from Zarrouk medium, diluted and sprayed again on fresh agar-containing plates. No fungal or algal contaminations were observed in the initial culture. To eliminate bacterial contamination, ampicillin or trimethoprim were used. While ampicillin did not prove effective, trimethoprim, a bacteriostatic antibiotic, at 5 - 30 µg·ml^{-1} was efficient to clean the cyanobacterial culture from contamination of other bacteria. Trimethoprim usually does not affect growth of cyanobacteria, such as *Synechocystis* sp. PCC 6803 or *Synechococcus elongatus* PCC 7942 ([26]; our unpublished observations), while inhibits *E. coli* and many other Gram-negative and Gram-positive bacterial species. Thus, we isolated the axenic cyanobacterial strain, which was deposited to the Collection of Microalgae and Cyanobacteria of the Institute of Plant Physiology RAS (Moscow, Russia) under accession number IPPAS B-1200.

3.2. Morphological and Structural Characterization of the Isolated Cyanobacterial Strain

Isolated colonies were blue-green, lustrous, circular, smooth, with straight edges (**Figure 1**). Light (**Figure 1**) and electron (**Figure 2**) microscopy of samples revealed the presence of cells of cyanobacteria resembling *Cyanobacterium* spp. of Chroococcales [27]. Cells were non-motile, solitary or in pairs after division, without

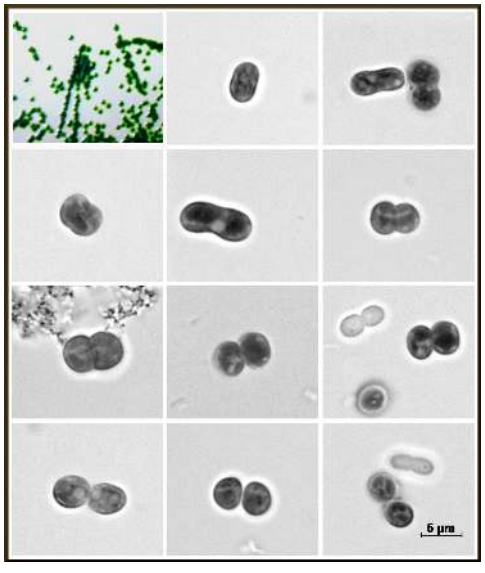

Figure 1. Colonies on agar and cells and of the isolated strain under light microscope.

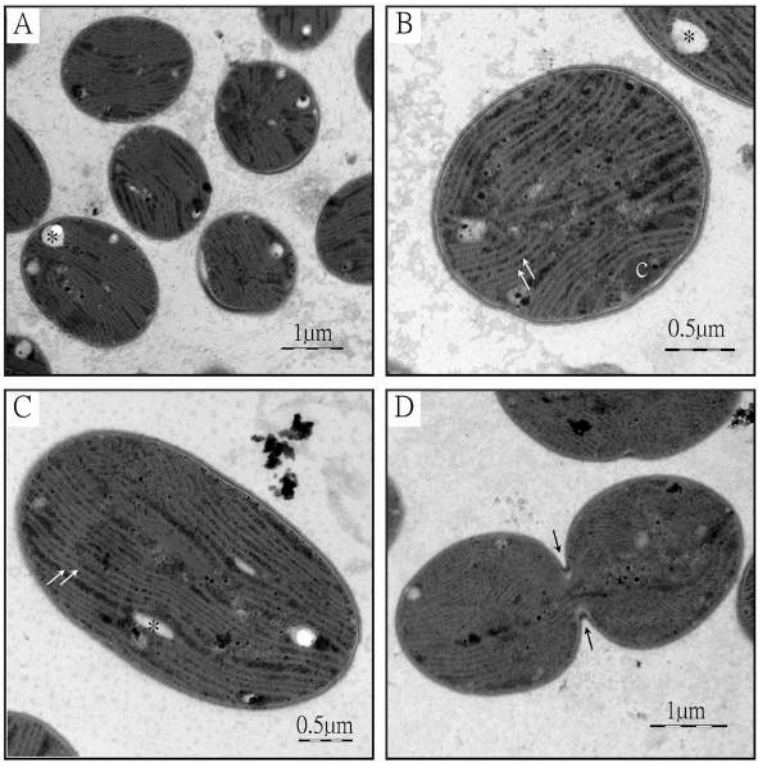

Figure 2. Electron microphotographs of cells of the isolated cyanobacterial strain: (A)—general view of cell population at low magnification; (B)—cross section of a cell with radial thylakoids; (C)—longitudinal section with lengthwise oriented thylakoids; (D)—dividing cells; c—carboxysomes; asterisks indicate inclusions with low electron density; white arrows mark thylakoid membranes; black arrows indicate invagination of cell wall in a dividing cell.

gelatinous envelopes, oval to rod-shaped with rounded ends, with average 4.5 - 7.5 μm length, 2.0 - 4.5 μm width, straight or slightly arcuate. Cell content had no separation on centro- and chromatoplasma. Cell division occurred by binary fission in transverse plane to longer cell axes and resulted in two isomorphic daughter cells (**Figure 1** and **Figure 2(D)**).

Ultrastructural analysis revealed radial thylakoids on cross-sections and lengthwise oriented thylakoids on longitudinal sections (**Figures 2(A)-(C)**). Few inclusion bodies were seen on sections: carboxysomes (**Figure 2(B)**) and inclusions with low electron density (**Figures 2(A)-(C)**). No S-layer or sheaths were observed on the outer membrane of cells.

Morphological and ultrastructural features of the isolated strain mostly fitted to the description of *Cyanobacterium stanieri* [27], which is considered to be a type strain of the genus *Cyanobacterium* [28]-[30]. The newly isolated strain differed from *Cyanobacterium stanieri* by bigger cell size (cells of the type strain are typically of 3.7 - 5.2 × 2 - 3.4 μm) and by the ability to excrete significant amount of mucilage, which was not attached to cells.

3.3. Growth Characteristics

A similar doubling time (9 - 11 h in the exponential phase) was observed during cultivation of the strain at 24˚C, 27˚C or 32˚C (**Figure 3**). At lower temperatures (24˚C - 27˚C), cultures had longer lag-phase, after which it could grow at a similar rate as at 32˚C. At high temperatures (39˚C), retardation of growth after the first day of incubation was observed (**Figure 3**). The ability of this strain to grow intensively in a rather wide temperature range is preferable for biotechnological applications, since it allows a deviation of daily cultivation temperature considerably. The absorption spectrum of cell suspension of the isolated strain was typical to unicellular cyanobacteria that contain chlorophyll *a* (not shown).

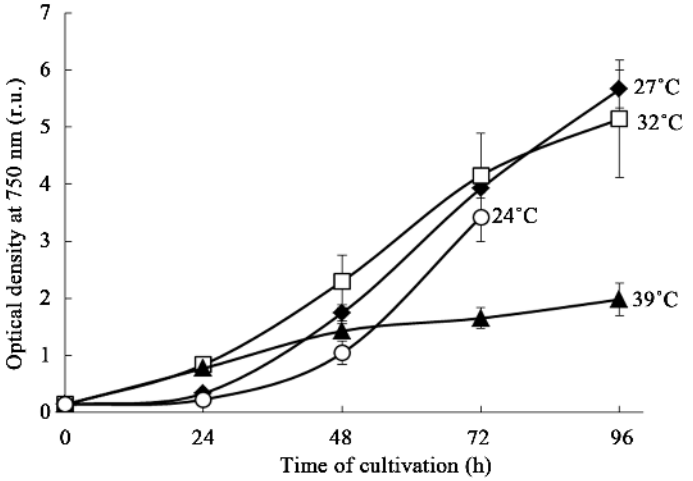

Figure 3. Growth curves of the isolated strain at different temperatures. Bars show standard deviations from 3 independent experiments. r.u.—reference units.

3.4. 16S DNA Cloning and Phylogenetic Analysis

The DNA fragment of 1488 bp that corresponded to the full-length 16S rRNA was amplified by PCR from the genomic DNA with a standard set of primers [22] as described in Materials and Methods. Amplified DNA fragments have been cloned into a T-type vector, and their nucleotide sequences were determined (GenBank Accession No. KM502966) (**Figure 4**).

The nucleotide sequence of DNA fragment that corresponded to the 16S small ribosomal RNA of the isolated cyanobacterium displayed the highest degree of homology to the 16S ribosomal RNA gene of *Cyanobacterium stanieri* strain PCC 7202 [31] (**Table 1**) or to some uncultured bacteria. The latter are, probably, the representatives of cyanobacterial species from environmental water samples that have been subjected to metagenomic analysis [32]. Thus, on the basis of microscopic and molecular studies, we assigned the newly isolated strain as *Cyanobacterium* sp. IPPAS B-1200.

3.5. Fatty Acid Composition of *Cyanobacterium* sp. IPPAS B-1200

Cells of *Cyanobacterium* sp. IPPAS B-1200 were grown at 32˚C or 24˚C until they reached the stationary growth phase. The isolated and transesterified FAMEs were resolved by GC-MS. Typical GC profile is represented in **Figure 5**. The cells of *Cyanobacterium* sp. IPPAS B-1200 accumulated up to 60 mg of FAs per gram of dry weight. The effect of temperature on FA composition was not observed. The cells were characterized by rather simple FA composition (**Table 2**) and contained equal amounts of saturated and monounsaturated (about 50% each) FAs. Palmitic (16:0) and palmitoleic (16:1Δ9) acids make 40% and 10%, respectively. C_{18} fatty acids are present in negligibly low amounts. The unique feature of *Cyanobacterium* sp. IPPAS B-1200 is that myristic (14:0) and myristoleic (14:1Δ9) acids are present in cells at high amounts –30% and 10%, respectively.

According to the proposed classification [9], the presence of only monounsaturated FAs assigns this strain to cyanobacteria of Group 1, which includes mesophilic and thermophilic species of unicellular cyanobacteria [9] [33]-[36].

4. Discussion

Fatty acid composition of lipids of cyanobacteria is conserved and it can be used as the phylogenetic marker [33] [35]. All cyanobacterial strains have been previously tagged into four distinct groups according to their FA composition [9].

The organisms of Group 1 synthesize only monoenoic fatty acids usually desaturated at Δ9 position. Recent advances in genomic sequencing and biochemical analysis reveal that such desaturation may be performed by different isozymes of the Δ9-desaturase. Some of these isozymes are specific to *sn*-1 or *sn*-2 positions of the

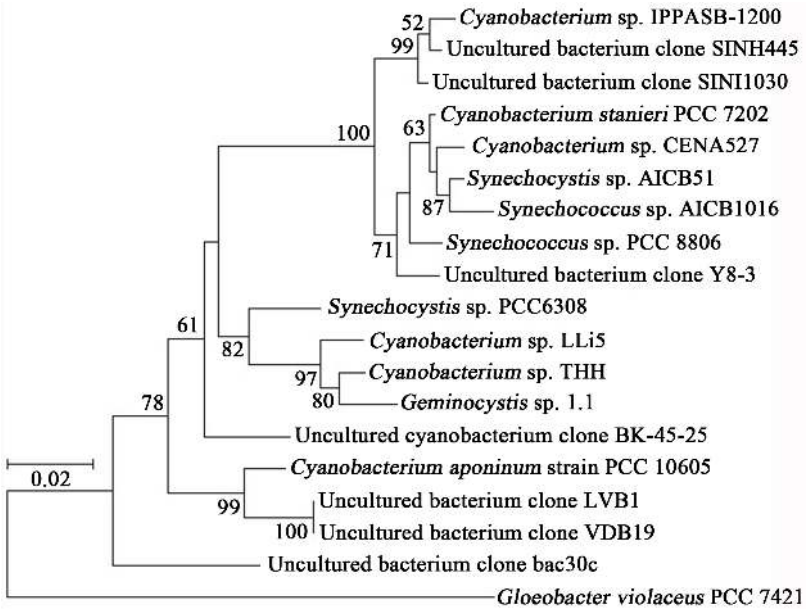

Figure 4. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences that produced highest homology score with GenBank sequence KM502966 (*Cyanobacterium* sp. IPPAS B-1200) and with *Gloeobacter violaceus* PCC 7421 (NR_074282) as an out-group. Bootstrap values > 50% are shown above branches. GenBank accession numbers are shown in **Table 1**.

Table 1. Nucleotide sequences that produced high alignment score.

Accession	Description	Max Score	Total Score	Query Cover	Identity
CP003940.1	*Cyanobacterium stanieri* PCC 7202, complete genome	2532	7319	99%	98%
HM128012.1	Uncultured bacterium clone SINH445 16S rRNA gene, partial	2516	2516	95%	99%
HM126696.1	Uncultured bacterium clone SINI1030 16S rRNA gene, partial	2508	2508	95%	99%
KF912998.1	Uncultured bacterium clone Y8-3 16S rRNA gene, partial	2431	2431	97%	97%
KF246493.1	*Cyanobacterium* sp. CENA527 16S rRNA gene, partial	2405	2405	95%	97%
AF448077.1	*Synechococcus* sp. PCC 8806 16S rRNA gene, partial	2398	2398	95%	97%
KJ746512.1	*Synechocystis* sp. AICB51 16S rRNA gene partial	2385	2385	95%	97%
KJ746511.1	*Synechococcus* sp. AICB1016 16S rRNA gene partial	2340	2340	96%	96%
FJ866623.1	Uncultured cyanobacterium clone BK-45-25 16S rRNA gene, partial	2148	2148	98%	93%
CP003947.1	*Cyanobacterium aponinum* PCC 10605, complete genome	2146	6403	99%	93%
DQ786164.1	*Cyanobacterium* sp. LLi5 16S rRNA gene, partial	2143	2143	98%	93%
KC621874.1	*Cyanobacterium* sp. THH 16S rRNA gene, partial	2137	2137	97%	93%
JX504279.1	Uncultured bacterium clone bac30c 16S rRNA gene, partial	2108	2108	99%	92%
KJ465919.1	Uncultured bacterium clone LVB1 16S rRNA gene, partial	2106	2106	97%	93%
KJ465958.1	Uncultured bacterium clone VDB19 16S rRNA gene, partial	2102	2102	97%	93%
AB039001.1	*Synechocystis* PCC 6308 gene for 16S rRNA, partial	2102	2102	97%	93%

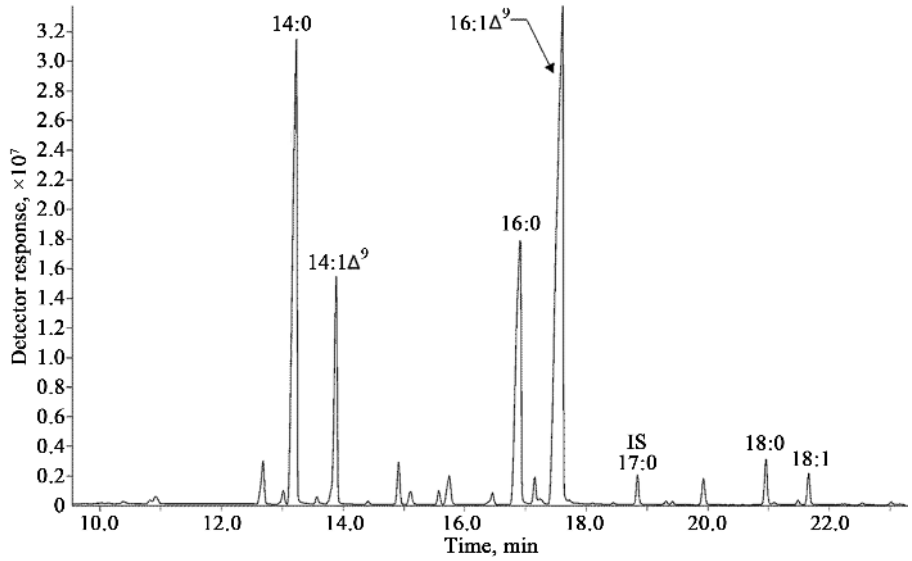

Figure 5. Typical profile of FAs separation by gas chromatography; IS—internal standard (17:0). Samples were prepared by direct transesterification.

Table 2. Fatty acid composition of *Cyanobacterium* sp. IPPAS B-1200.

Fatty Acids	Content of Fatty Acids, mol %[a]	
	32˚C	24˚C
14:0	29	30
14:1Δ9	9	10
16:0	20	17
16:1Δ9	39	40
18:0	1	1
18:1[*]	2	2

[a]The deviation of values was within 0.1% - 1%; [*]Sum of 18:1Δ9 and 18:1Δ11.

glycerol moiety [35]. The presence of five genes for the Δ9-desaturases in the genome of *Gloeobacter violaceus* [37] suggests that some isozymes may be also specific to the carbon chain length of FAs. On the other hand, the presence of only one gene for the Δ9-desaturase in the genomes of *Synechococcus elongatus* PCC 7942 (NCBI Reference Sequence NC_007604) or 6301 (NC_006576) and the appearance of 18:1 and 16:1 at *sn*-1 and *sn*-2 positions [9] suggest that some of Δ9-desaturases may be not as specific as others.

The cyanobacterial strains of Group 2 have three distinct FA desaturases and can synthesize trienoic α-linolenic acid, 18:3Δ9,12,15.

Organisms of Group 3 also have three distinct desaturases, but, instead of Δ15, they introduce a double bond at position Δ6 and produce trienoic γ-linolenic acid, 18:3Δ6,9,12, as a final product of desaturation.

Strains of Group 4 have four desaturases and synthesize tetraenoic stearidonic acid, 18:4Δ6,9,12,15.

Nowadays, a new group of marine cyanobacteria has emerged that has two FA desaturases, Δ9- and Δ12-desaturases [10], and that can synthesize dienoic 16:2Δ9,12 and 18:2Δ9,12 FAs.

The identified strain, *Cyanobacterium* sp. IPPAS B-1200, belongs to Group 1, and it can produce only saturated and monounsaturated fatty acids (**Table 2**). To our knowledge, this is the first characterized cyanobacterial strain that possesses very high amounts of C_{14} FAs: 14:0 (30%) and 14:1Δ9 (10%).

The chlorophyll *b* containing representative of Prochlorales, *Prochlorothrix hollandica*, which is known as a C_{14}-rich organism, possesses 5% of 14:0 and 30% of 14:1Δ9 [15]. *Prochlorothrix*, also with Δ9-desaturase, has the unique Δ4-desaturase activity and produces unusual 16:1Δ4 (25%) and 16:2Δ4,9 (10%) FAs [15]. The ge-

netic data for the cyanobacterial Δ4-desaturase is still unavailable. Nevertheless, the presence of high amounts of 16:2Δ4,9 (and the complete absence of 18:2 FAs) should place *Prochlorothrix hollandica* to an additional specialized Group of cyanobacteria classified according to their FA composition.

Phylogenetic analysis based on 16S rRNA sequence reveals that the closest relative to the newly identified strain *Cyanobacterium* sp. IPPAS B-1200 is *Cyanobacterium stanieri* PCC 7202 [38]. Among two putative genes annotated as fatty acid desaturases in the genome of the *Cyanobacterium stanieri* PCC 7202 (Accession number CP003940 [31]), one gene, AFZ46762 (Cyast_0790), encodes the *β*-carotene hydroxylase, but not fatty acid desaturase. The other gene, YP_007164144 (Cyast_0516), encodes the real Δ9-acyl-lipid desaturase. Therefore, FAs of *Cyanobacterium stanieri* PCC 7202 should be represented only by saturated and monounsaturated components, and *Cyanobacterium stanieri* PCC 7202 should belong to cyanobacteria of Group 1. However, experimental data on FA composition of that strain are missing.

Cyanobacterium stanieri PCC 7202 was originally classified as *Synechococcus* sp. PCC 7202. Later, it was separated from *Synechococcus* into a new genus due to morphological, biochemical and genetic differences [28] [39]. The original reference strains of fresh-water *Synechococcus*, *Anacystis nidulans* PCC 6301 and *Synechococcus* sp. PCC 7942, have been renamed to *Synechococcus elongatus* PCC 6301 and *Synechococcus elongatus* PCC 7942, respectively. These two genetically and closely related strains belong to Group 1. They possess one Δ9-desaturase gene, and they are able to synthesize only monounsaturated FAs. In this aspect, the affiliation of some marine species, which belong to Group 3 (*Synechococcus* sp. PCC 7002 that synthesizes trienoic FAs) or to a newly selected group (organisms that synthesize dienoic FAs), with the genus *Synechococcus*, looks somewhat questionable.

Morphology and ultrastructure of *Cyanobacterium* sp. IPPAS B-1200 are closer to *Cyanobacterium stanieri* than to *Synechococcus elongatus* (**Figure 1** and **Figure 2**). In the NCBI taxonomy database, *Cyanobacterium stanieri* PCC 7202 (Taxonomy ID: 292563) is placed together with its synonyms: *Synechococcus* sp. PCC 7202 and *Synechococcus cedrorum* CCAP 14792. *Synechococcus cedrorum* (no genomic data are available) is characterized by the presence of only monounsaturated FAs (16:0 ~ 47%, 16:1 ~ 39%, and 18:1 ~ 10%) [33] [34]. The shape and morphological features of *Synechococcus cedrorum* resemble that of *Synechococcus elongatus* [34], but not *Cyanobacterium stanieri* PCC 7202 [28]. The origin and systematic position of *Synechococcus cedrorum* were already questioned in the previous century [28]; however, it seems that the problem still remains unsolved.

Thus, we have isolated, from Lake Balkhash, Republic of Kazakhstan, purified and identified a new representative of unicellular cyanobacteria, which was named *Cyanobacterium* sp. IPPAS B-1200, according to its morphological characteristics, 16S rRNA analysis and FA composition. Lipids of this strain are rich in C_{14} myristic and myristoleic acids (30% and 10%, respectively) and in C_{16} palmitic and palmitoleic acids (20% and 40%, respectively). This simple and unusual FA composition makes this strain a good candidate for synthesis of the carbon chains suitable for biodiesel production.

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